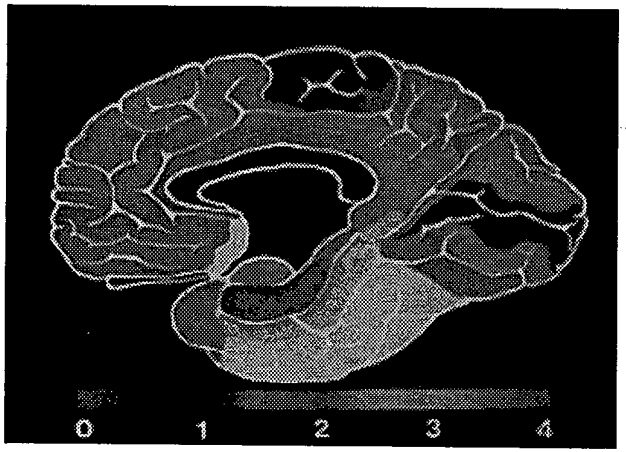
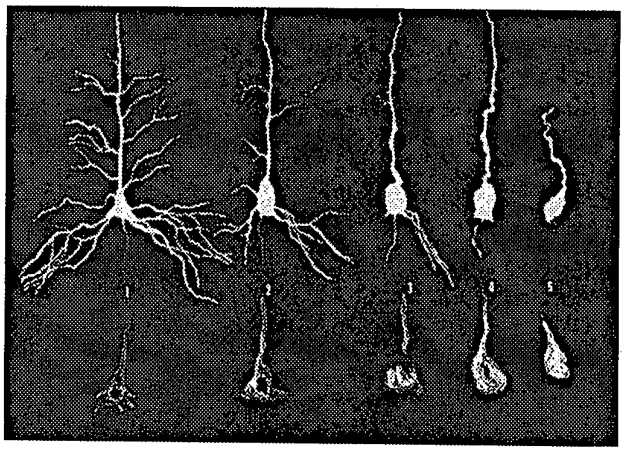
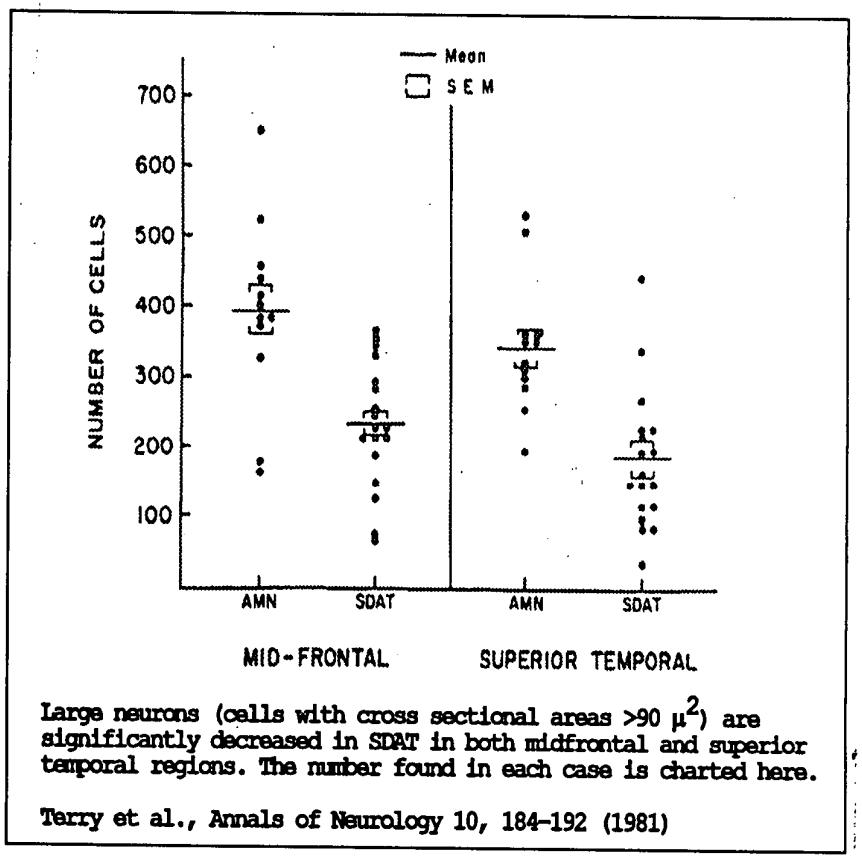
TOSTOT"h52FE860

Selective vulnerability of brain regions in Alzheimer´s disease







Large neurons (cells with cross sectional areas >90 μ^2) are significantly decreased in SDAT in both midfrontal and superior temporal regions. The number found in each case is charted here.

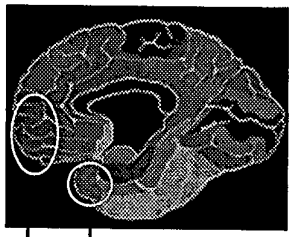
Terry et al., Annals of Neurology 10, 184-192 (1981)

Figure 1

09/831754

T0ST0T"4S27E860

Identification of genes differentially expressed in AD brain regions

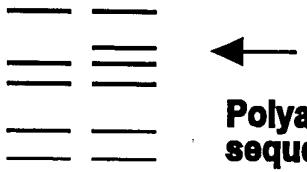


extract RNA

RT | oligo-dT-primer

cDNA pool

PCR | random primer
oligo-dT primer
α^{35}S dATP



Polyacrylamide-sequencing-gel

- cut out differentially expressed bands
- reamplify eluted cDNAs
- cloning and sequencing
- expression analysis
- functional analysis

Figure 2

09/831754

T05T0T"1S2TE860

Identification of genes differentially expressed in AD brain regions

Material: AD brain tissue

post mortem time intervall <6h

2 different regions histologically characterized

- inferior temporal lobe
- frontal cortex

Method: mRNA differential display screen

Figure 3

09/831754

T0STOT"4S2T2860

Expression of Seladin-1 in AD brain

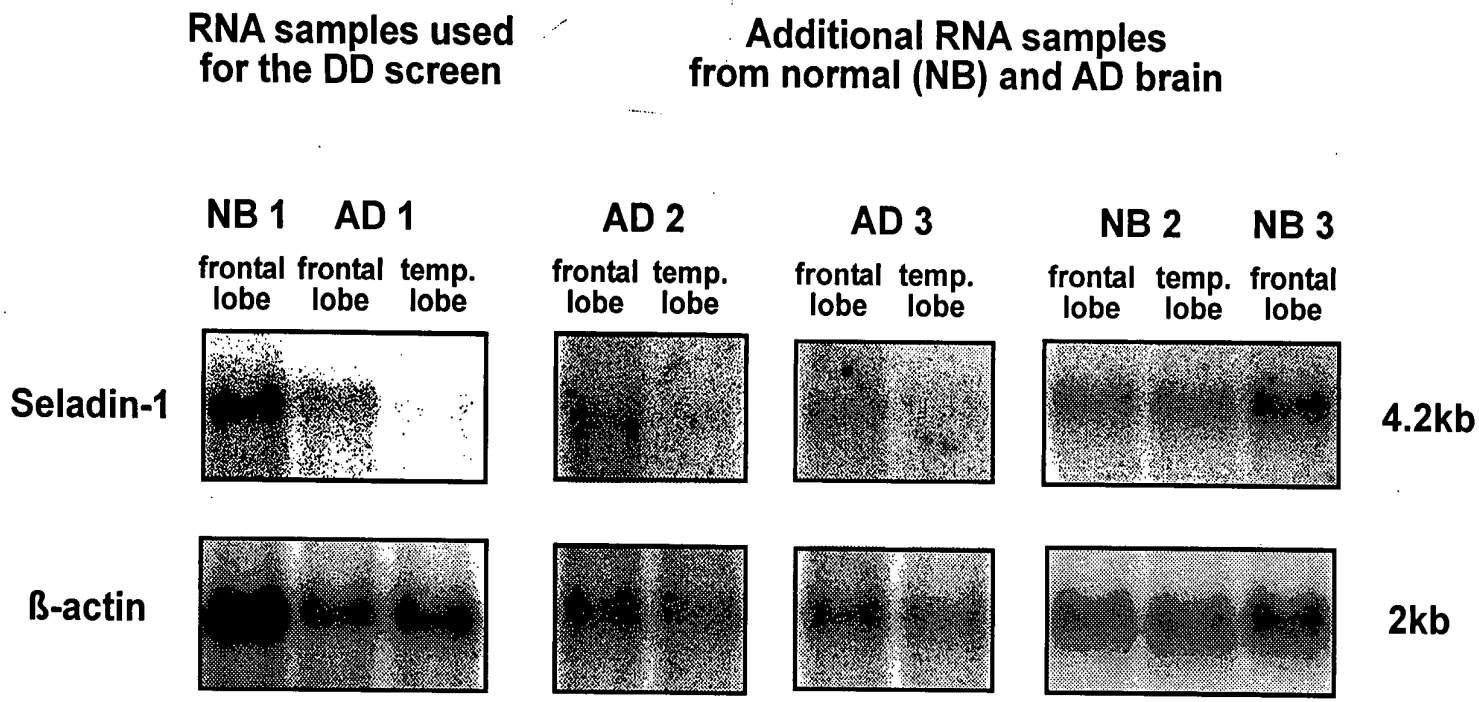


Figure 4

09/831754

TOSTOT"tSZTE860

Expression of Seladin-1 in different human brain regions

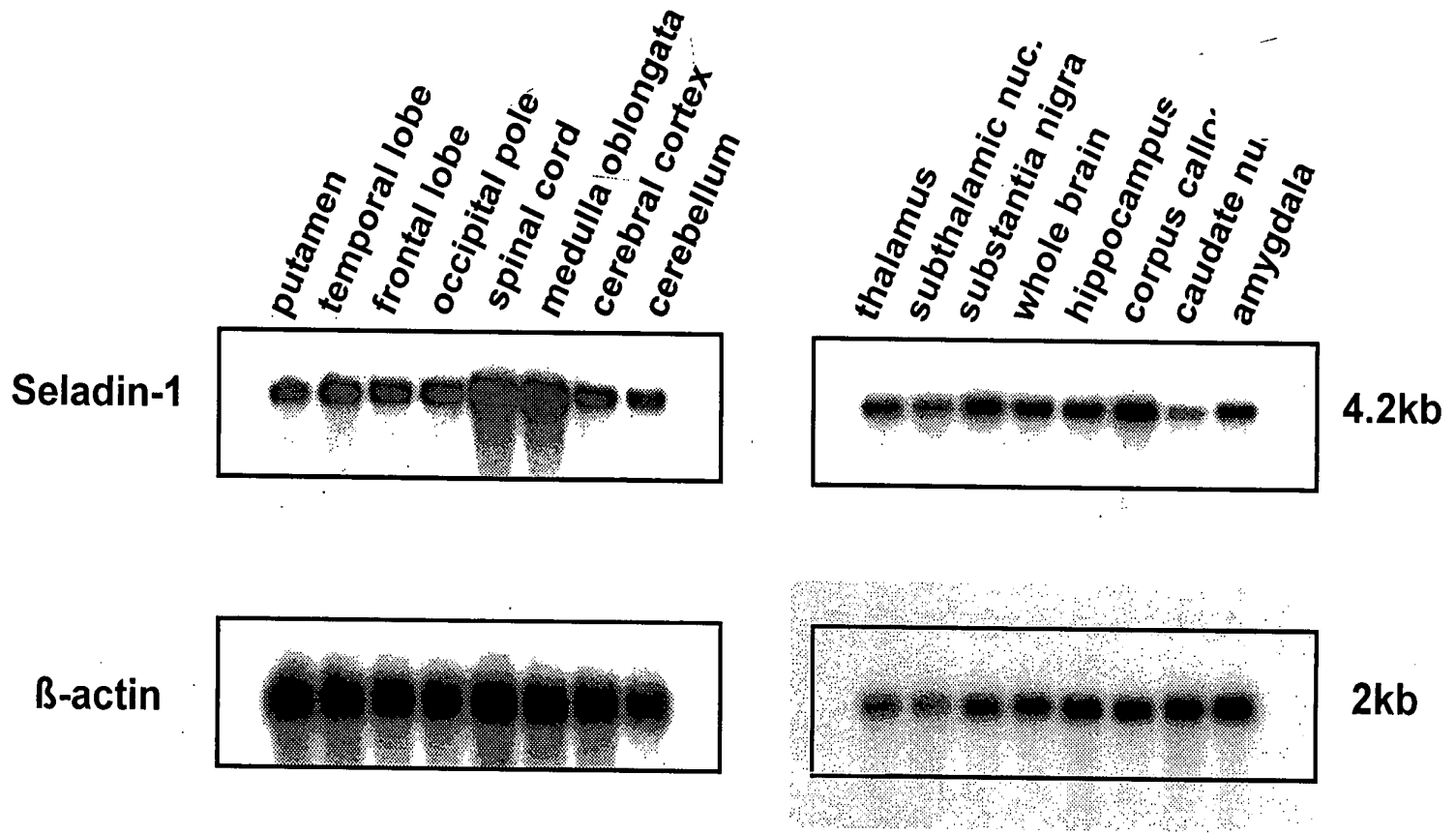
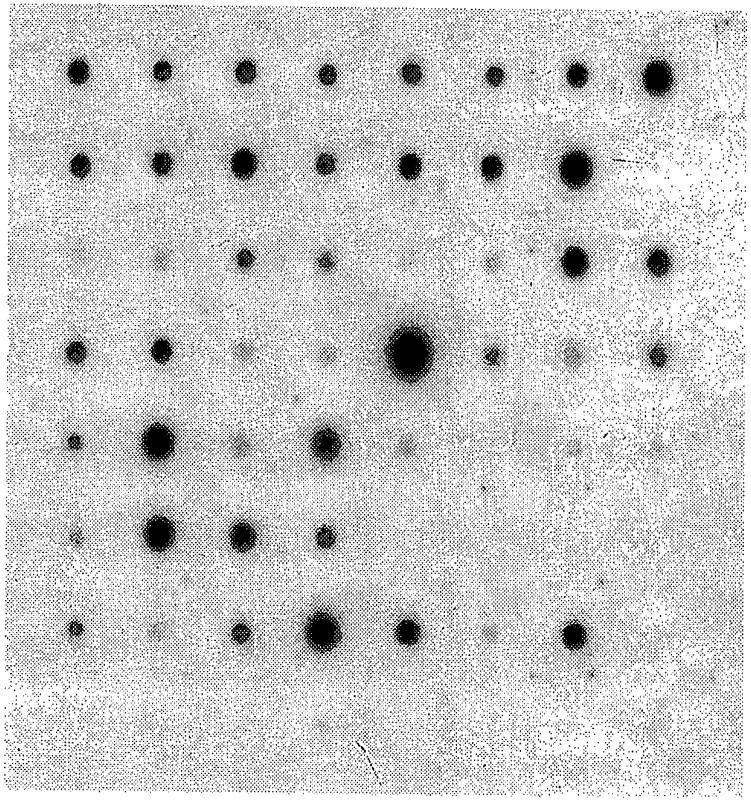


Figure 5

09/831754

TOSTOT "hSZTE860

Expression of Seladin-1 in human tissues



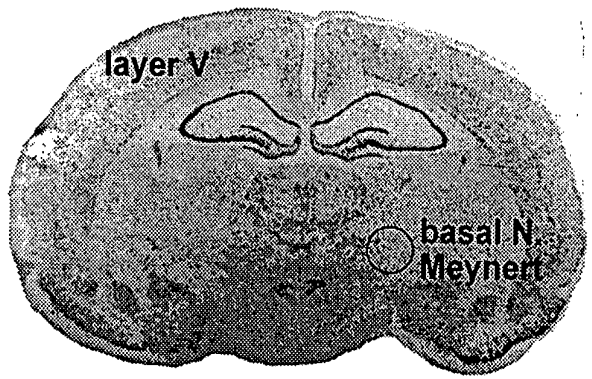
whole brain	amyg-dala	caudate nucleus	cere-bellum	cerebral cortex	frontal lobe	hippo-campus	medulla oblong-ata
occipital lobe	putamen	sub-stantia nigra	temporal lobe	thalamus	sub-thalamic nucleus	spinal cord	
heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mam-mary gland
kidney	liver	small intestine	spleen	thymus	periphe-ral leuko-cyte	lymph node	bone marrow
appendix	lung	trachea	placenta				
fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
yeast total RNA 100ng	yeast tRNA 100ng	E. coli rRNA 100ng	E. coli DNA 100ng	Poly r(A) 100ng	human Cot1DNA 100ng	human DNA 100ng	human DNA 500ng

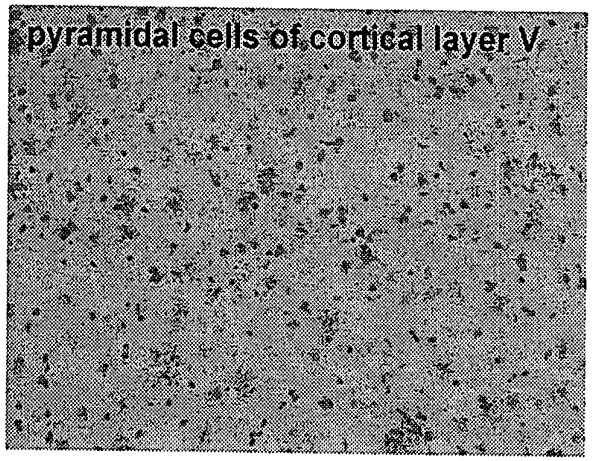
Figure 6

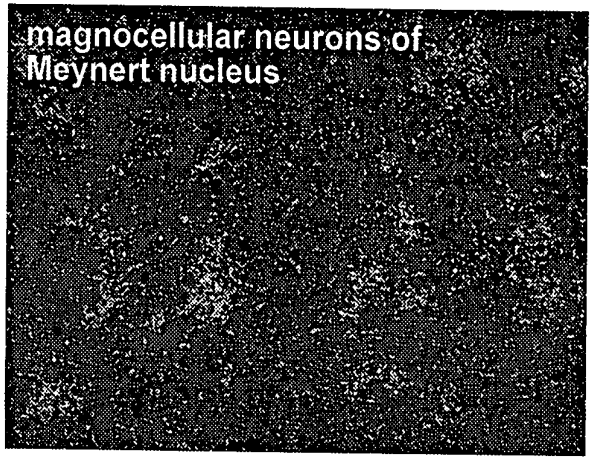
09/831754

TOSTOT "hSZtE860

Expression of Seladin-1 in rat brain







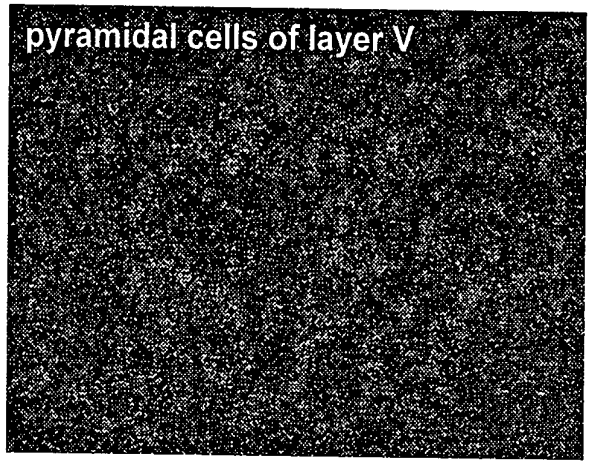


Figure 7

09/831754

TOSTOT "hSZTE860

Expression of Seladin-1 in rat brain

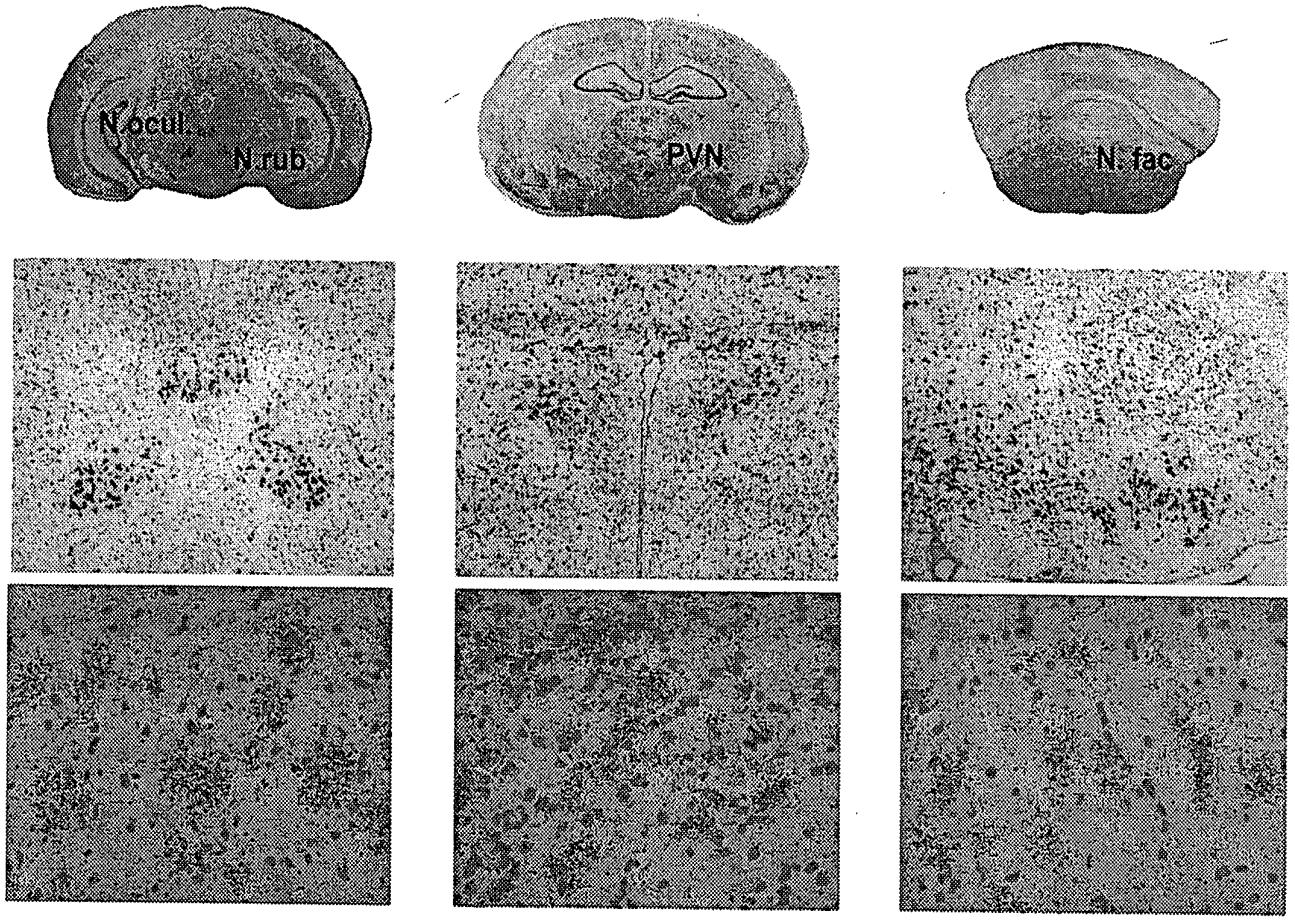
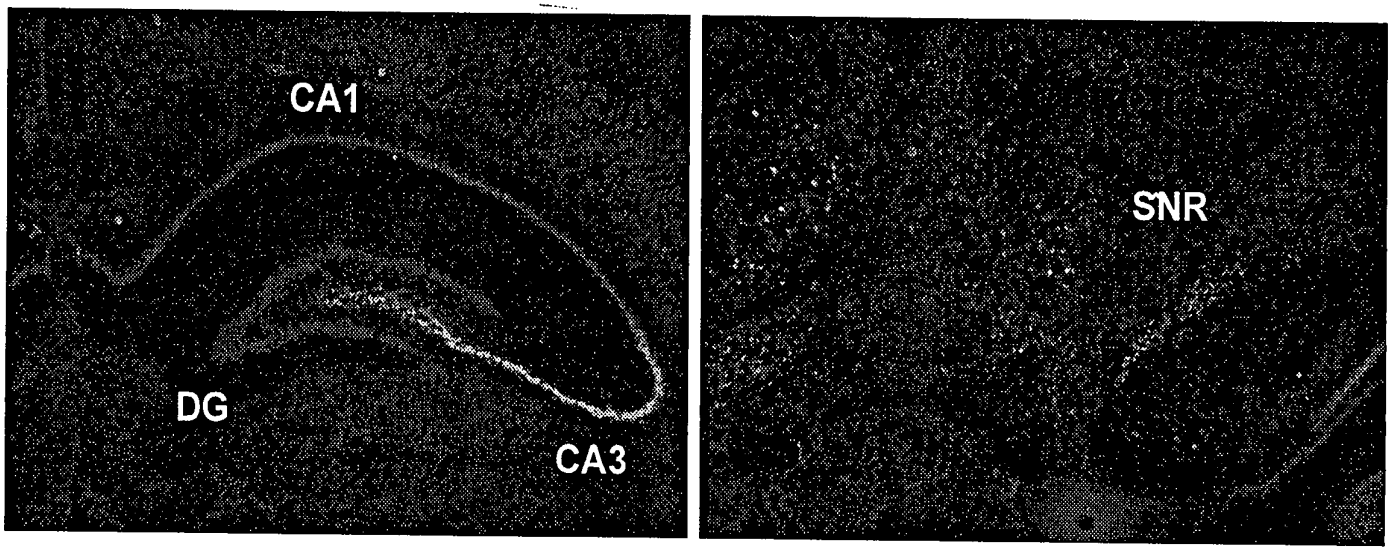


Figure 8

09/831754

TOSTOT"hSZTE860

Expression of Seladin-1 in rat brain



hippocampus

substantia nigra

Figure 9

09/831754

TOSTOT"tSZtE860

Subcellular localization of Seladin-1 EGFP fusionprotein

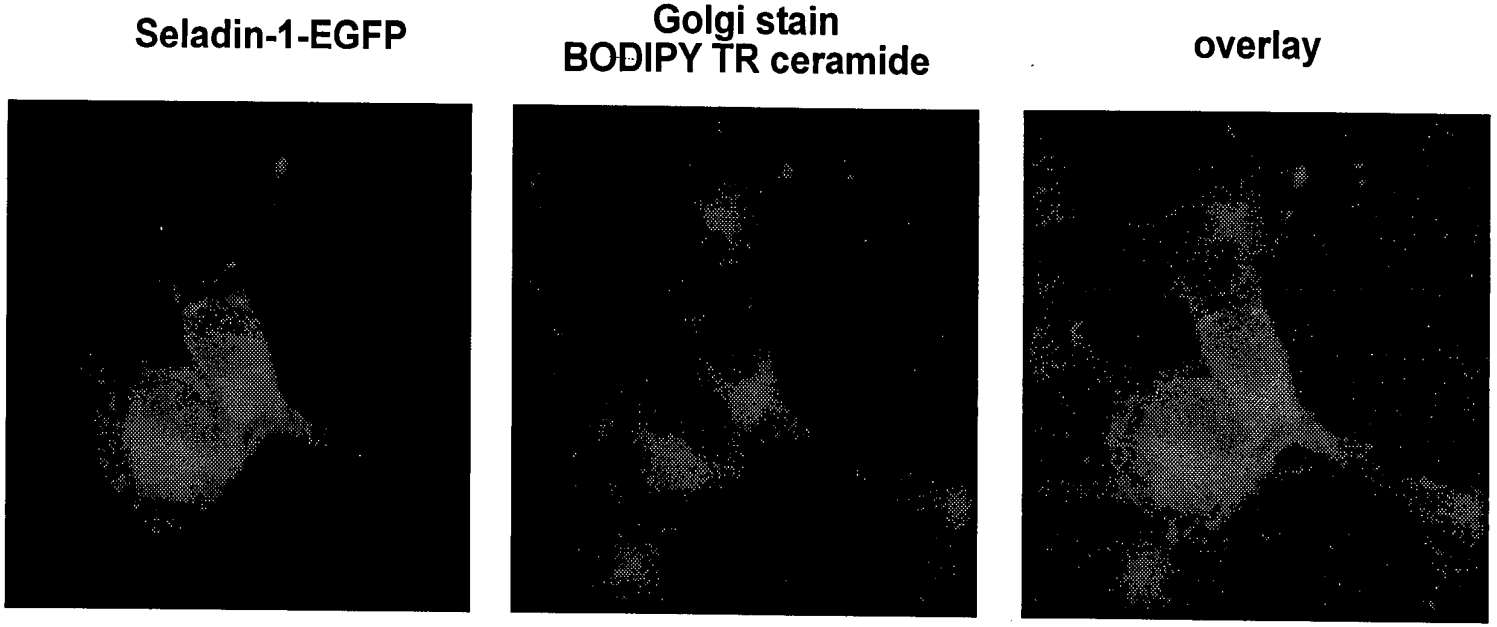


Figure 10

09/831754

T05T0T' tS/TE860

Subcellular localization of Seladin-1 EGFP fusionprotein

Seladin-1-EGFP

Mitochondrial stain
MitoTracker Red CM-H2XRos

overlay

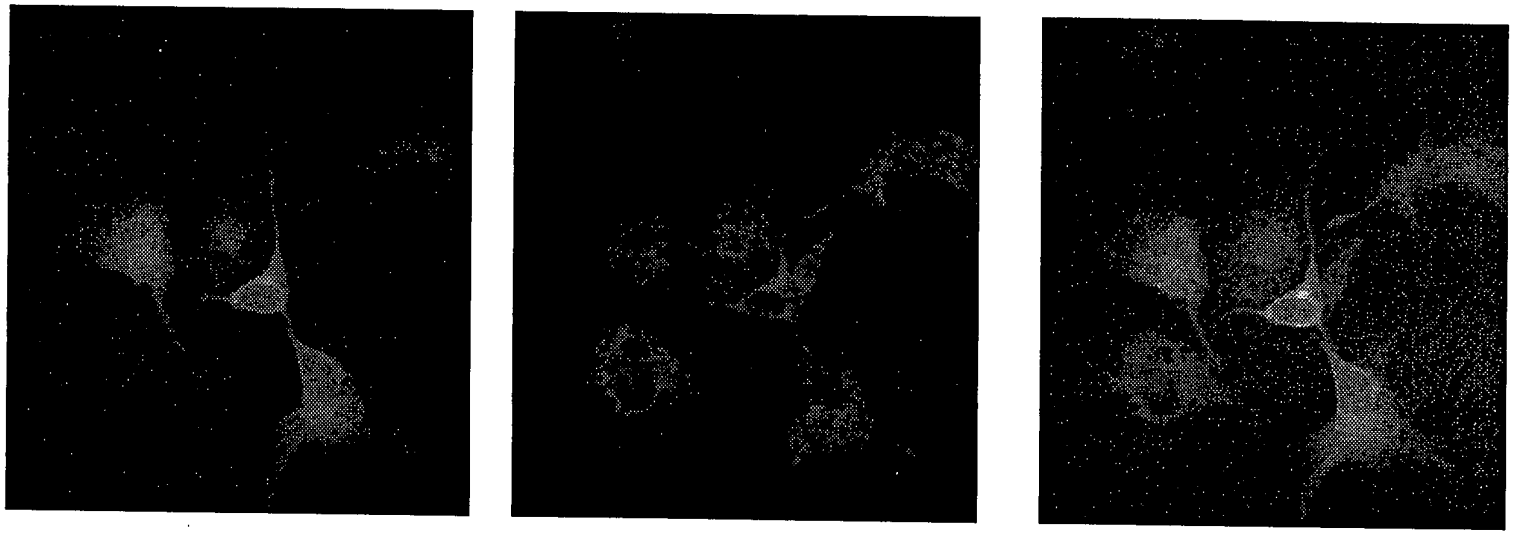


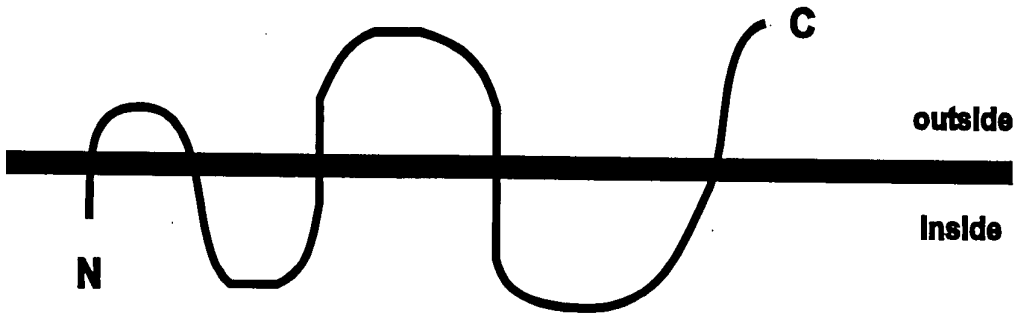
Figure 11

09/831754

TOSTOT "4527E860

Multiple sequence alignments and secondary structure prediction of Seladin-1

- putative mitochondrial targeting sequence
- homology region to many oxido-reductases
  e.g. L-gulono-gamma-lactone-oxidase
  6-hydroxy-d-nicotine oxidase
  D-lactate dehydrogenase
- contains oxygen oxidoreductases covalent FAD binding site



- 5 possible transmembrane regions

Figure 12

09/831754

FIGURE 13: SEQ ID NO.1

Seladin-1 amino acid sequence

Seladin-1.orf Length: 516 May 29, 1998 14:51 Type: P
Check: 1354 ..

```
1    MEPAVSLAVC ALLFLLWVRL KGLEFVLIHQ RWVFVCLFLL PLSLIFDIYY
51   YVRAWVVFKL SSAPRLHEQR VRDIQKQVRE WKEQGSKTFM CTGRPGWLTV
101  SLRVGKYKKT HKNIMINLMD ILEVDTKKQI VRVEPLVTMG QVTALLTSIG
151  WTLPVLPELD DLTVGGLIMG TGIESSSHKY GLFQHICTAY ELVLADGSFV
201  RCTPSENSDL FYAVPWSCGT LGFLVAAEIR IIPAKKYVKL RFEPVRGLEA
251  ICAKFTHESQ RQENHFVEGL LYSLDEAVIM TGVMTDEAEP SKLNSIGNYY
301  KPWFFKHVEN YLKTNREGLE YIPLRHYYHR HTRSIFWELQ DIIPFGNNPI
351  FRYLFGWMVP PKISLLKLTQ GETLRKLYEQ HHVVQDMLVP MKCLQQALHT
401  FQNDIHVYPI WLCPFILPSQ PGLVHPKGNE AELYIDIGAY GEPRVKHFEA
451  RSCMRQLEKF VRSVHGFQML YADCYMNREE FWEMFDGSLY HKLREKLGCQ
501  DAFPEVYDKI CKAARH
```

DOCID: <WO 9855504A1>

FIGURE 14: SEQ ID NO.2

Seladin-1 cDNA sequence

Seladin-1 Length: 4248 April 28, 1998 14:10 Type: N Check: 8184 ..

```
   1  cccgggctgt gggctacagg cgcagagcgg gccaggcgcg gagctggcgg
  51  cagtgacagg aggcgcgaac ccgcagcgct taccgcgcgg cgccgcacca
 101  tggagcccgc cgtgtcgctg gccgtgtgcg cgctgctctt cctgctgtgg
 151  gtgcgcctga aggggctgga gttcgtgctc atccaccagc gctgggtgtt
 201  cgtgtgcctc ttcctcctgc cgctctcgct tatcttcgat atctactact
 251  acgtgcgcgc ctgggtggtg ttcaagctca gcagcgctcc gcgcctgcac
 301  gagcagcgcg tgcgggacat ccagaagcag gtgcgggaat ggaaggagca
 351  gggtagcaag accttcatgt gcacggggcg ccctggctgg ctcactgtct
 401  cactacgtgt cgggaagtac aagaagacac acaaaaacat catgatcaac
 451  ctgatggaca ttctggaagt ggacaccaag aaacagattg tccgtgtgga
 501  gcccttggtg accatgggcc aggtgactgc cctgctgacc tccattggct
 551  ggactctccc cgtgttgcct gagcttgatg acctcacagt ggggggcttg
 601  atcatgggca caggcatcga gtcatcatcc cacaagtacg gcctgttcca
 651  acacatctgc actgcttacg agctggtcct ggctgatggc agctttgtgc
 701  gatgcactcc gtccgaaaac tcagacctgt tctatgccgt accctggtcc
 751  tgtgggacgc tgggtttcct ggtggccgct gagatccgca tcatccctgc
 801  caagaagtac gtcaagctgc gtttcgagcc agtgcggggc ctggaggcta
 851  tctgtgccaa gttcacccac gagtcccagc ggcaggagaa ccacttcgtg
 901  gaagggctgc tctactccct ggatgaggct gtcattatga caggggtcat
 951  gacagatgag gcagagccca gcaagctgaa tagcattggc aattactaca
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1251  gcaggacatg ctggtgccca tgaagtgcct gcagcaggcc ctgcacacct
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1401 catcgacatt ggagcatatg gggagccgcg tgtgaaacac tttgaagcca
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2251 ctgtgccaaa gccaggtccg aggccaaagt tctccctgcc atccttggtg
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2601 aggtgggaaa gtggagtcca ccctcatgtt tctgtaggat tctcaccgtg
2651 gggctggaag aaaagagcat cgacttgatt tctccaacca ctcatccctc
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DOCUMENT - 09831754

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3101 cttctgattg tcttagacac tgtggttgca aaacccacgg aaagcctcat

3151 ttgtgtggaa agtcagagga aaaatgatcc agtggacact tggggattat

3201 ctgtcattca agatccttcc ttcaacccca aggccagctc ccatctcatt

3251 tccagaaagg ctcatacctg gcttgcaggg aagcatctgt cttgtcattc

3301 caggtgccag aatcctctca gagtcattga agggtgttca cccatcccac

3351 ccaaggcttg gcacactgcc agtgtcttag cagggtcttg tgagggctgg

3401 gggcatccag gcactcagaa ggcaaaggaa ccaccctacc catttggcct

3451 ctggaggggg cagaagaaag aaagaaacct catcctatat tttacaaagc

3501 atgtgaattc tggcattagc tctcatagga gacccatgtg cttccttgct

3551 cagtgcaaaa ctgatgattc tacttgctgt agatgaatgg ttaacacgag

3601 ctagttaaac agtgccattg ttttgccagt gaagcctcca accctaagcc

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3751 ttttggaatc acaccctccg gttatgttgc tccagtaaaa tcttgcctgg

3801 aaagaggcag tcttcttagc atggtgagct gagttcatgg ctttttttg

3851 tagccagtcc tgtccctggc catccatgtg atggttttgg atggagttaa

3901 acttgatgcc agtgggcagt gcatgtggaa agtatcagag taagcctctc

3951 ccctccagag ccctgagttt cttggctgca tgaaggtttt ctttagaatc

4001 agaattgtag ccagtttctt tggccagaag gatgaatact tggatattac

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4151 gtcaggctct tacagccaca ggcactgtgt ctactgtctg gaagacatgt

4201 ccccgtggct gtggggccgc tgcttctgtt taaataaaag tggcctgg

Doc#: 1050354 v1

FIGURE 15 CDNA sequence comparison KIAA0018/Seladin-1

```
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     ||||||||||||||||||||||||||||||||||||||||||||||||||
  62 ggcgcgaacccgcagcgcttaccgcgcggcgccgcaccatggagcccgcc 111

  51 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 112 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 161

 101 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 150
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 162 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 211

 151 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 200
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 212 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 261

 201 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 250
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 262 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 311

 251 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 300
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 312 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 361

 301 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 350
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 362 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 411

 351 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 400
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 412 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 461

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     ||||||||||||||||||||||||||||||||||||||||||||||||||
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```

09831754 - 101500

```
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    ||||||||||||||||||||||||||||||||||||||||||||||||||
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851 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 900
    ||||||||||||||||||||||||||||||||||||||||||||||||||
912 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 961

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Fig. 16

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151  WTLPVLPELD DLTVGGLIMG TGIESSSHKY GLFQHICTAY ELVLADGSFV
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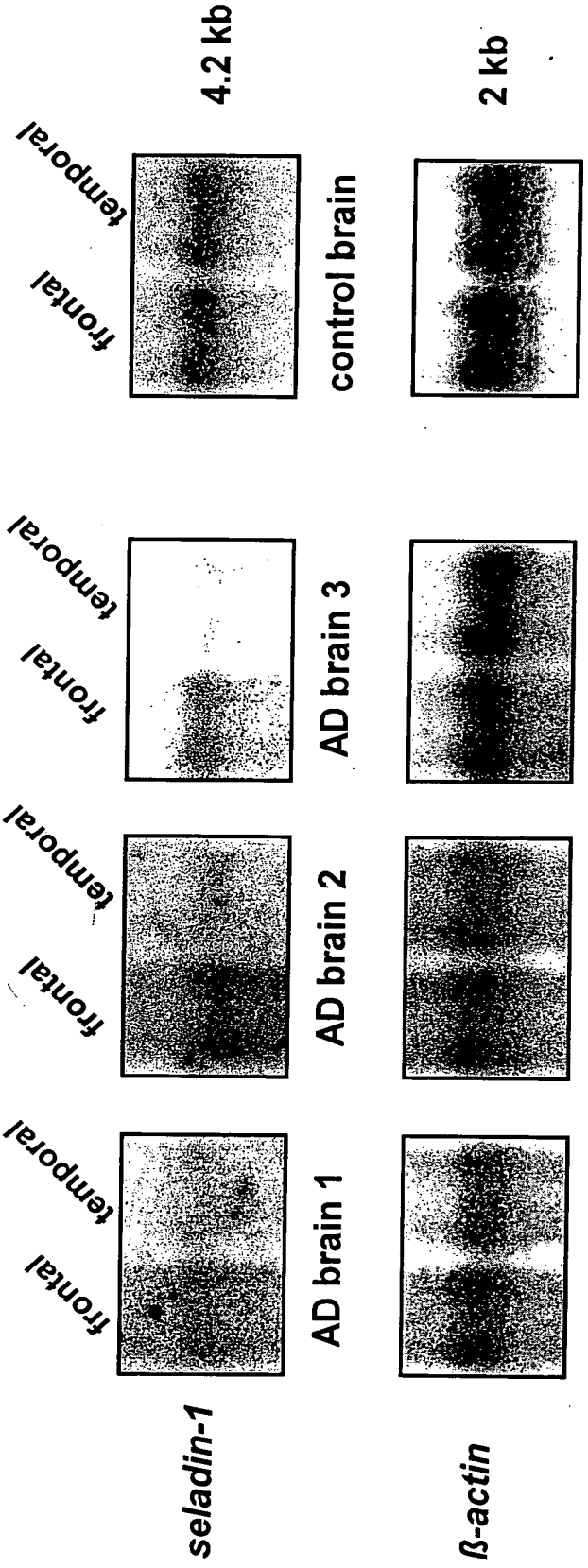

Fig. 17 A

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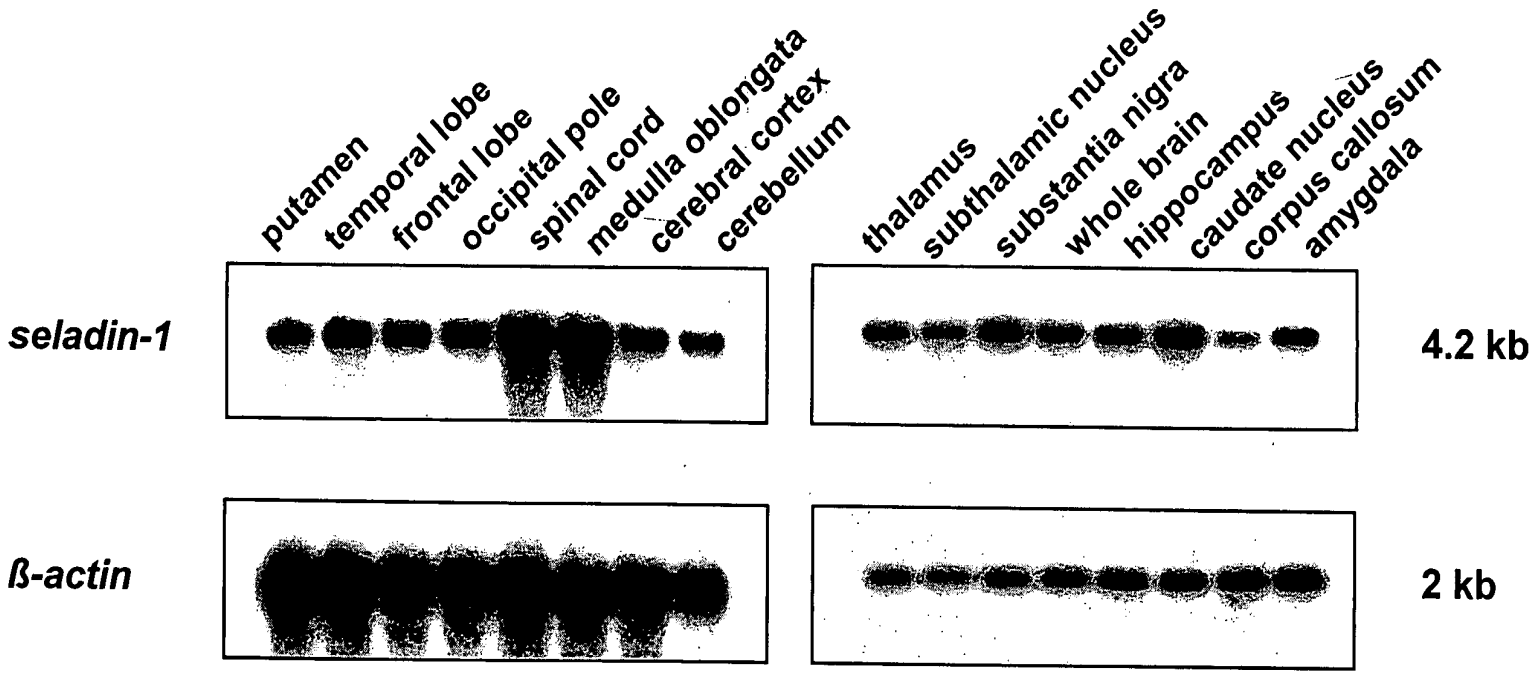

Fig. 17 B

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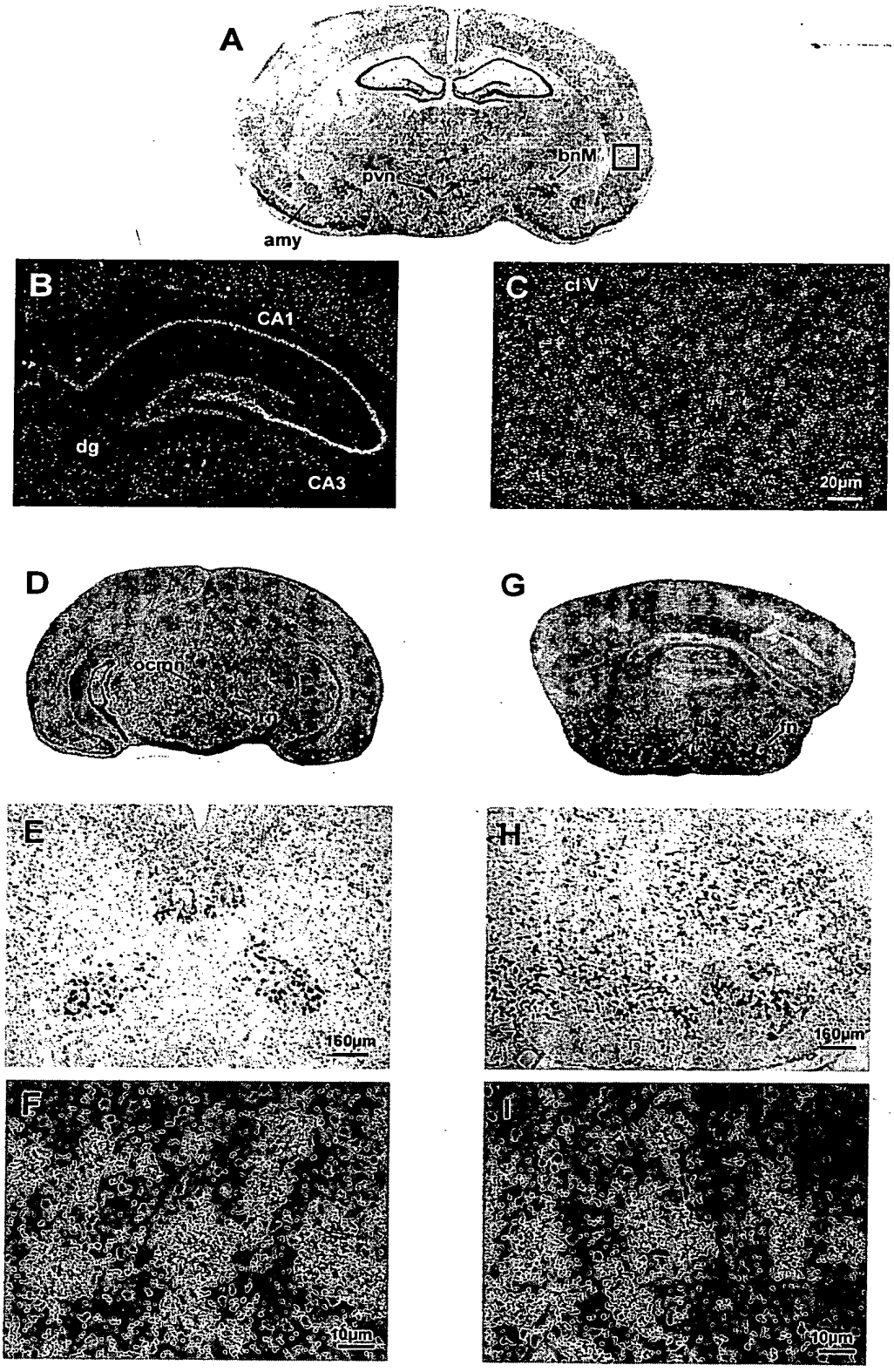

Fig. 18

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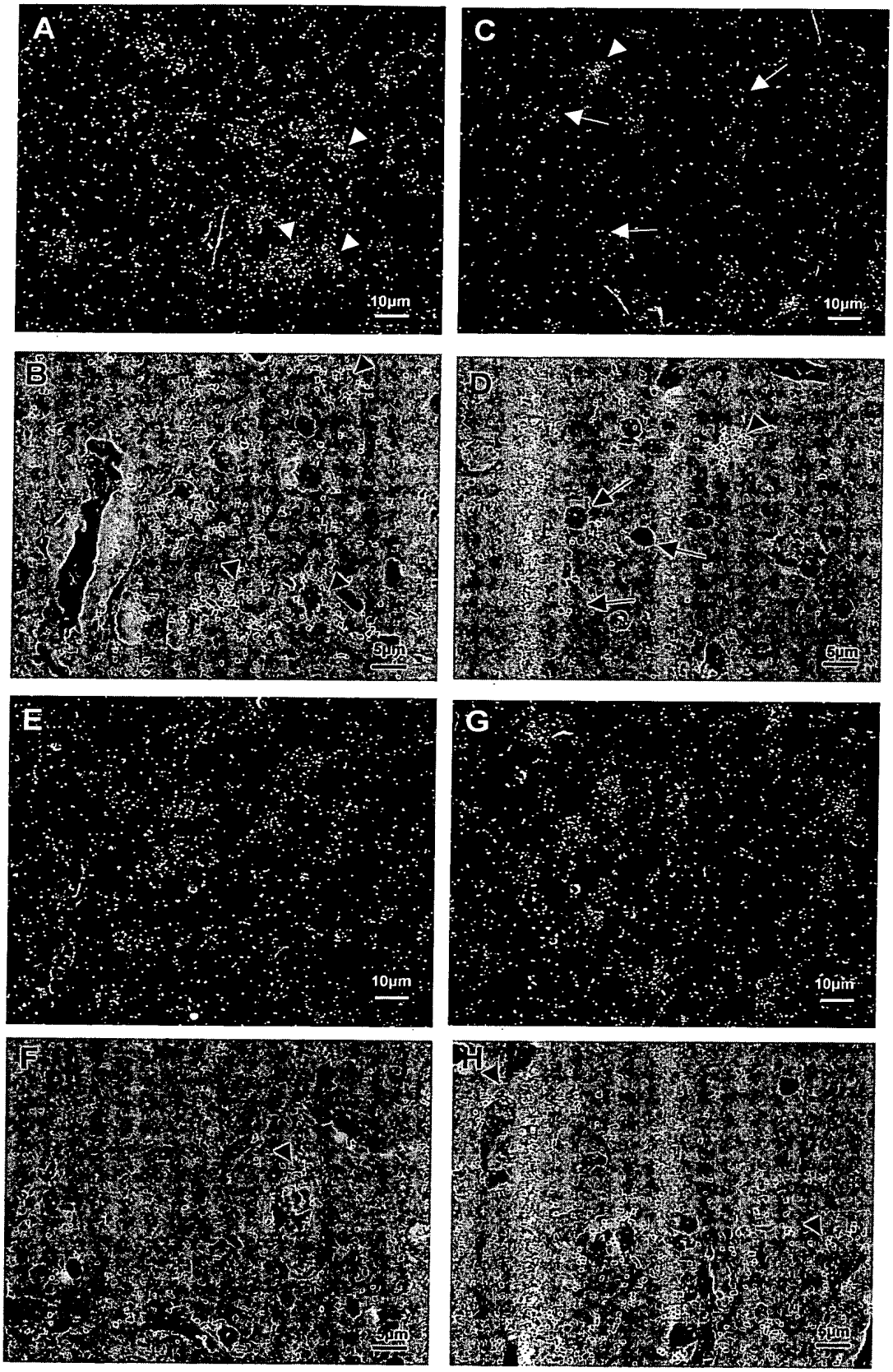

Fig. 19

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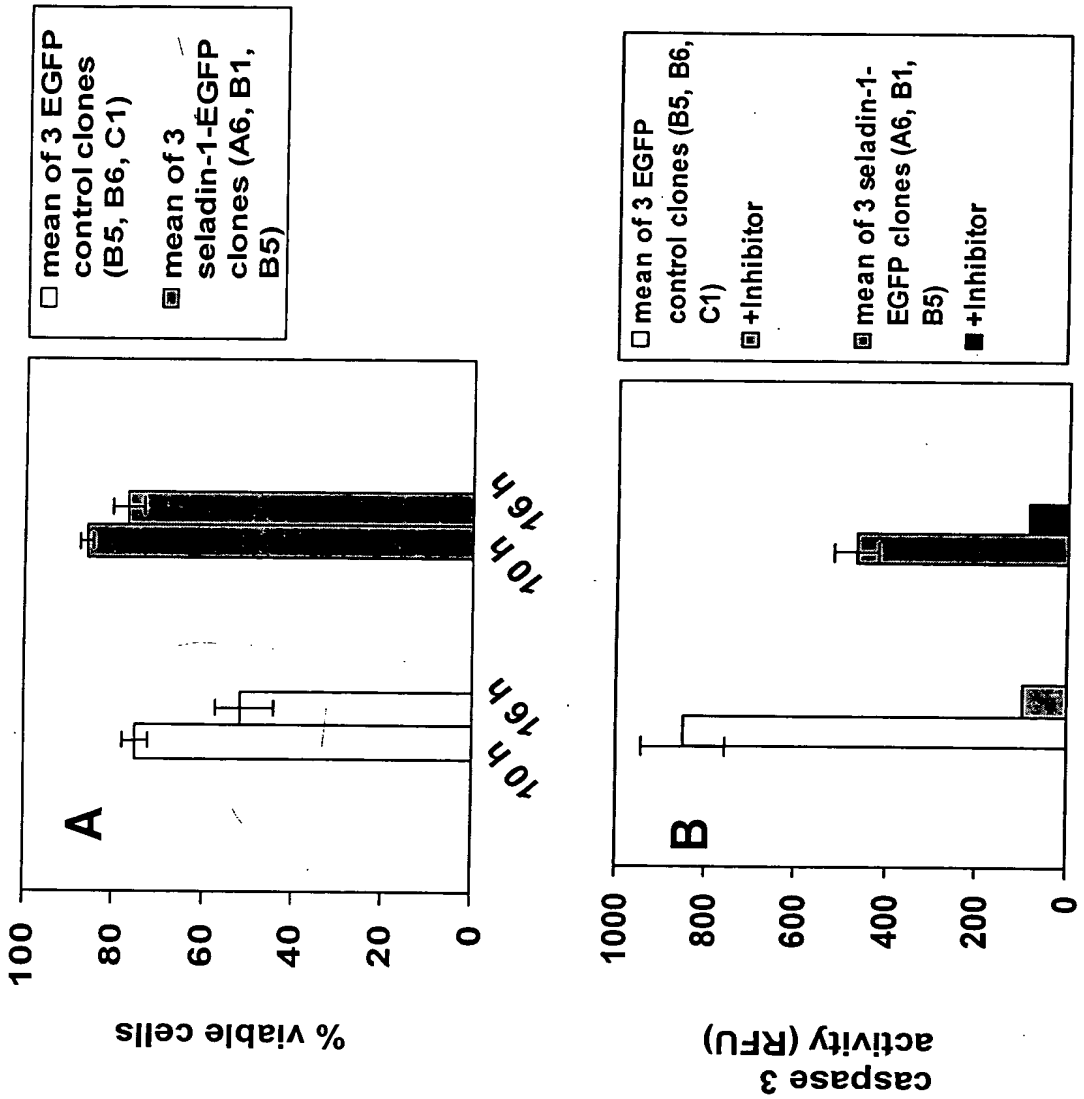
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% viable cells
100
80
60
40
20
0
10h
16h
10h
16h
mean of 3 EGFP control clones (B5, B6, C1)
mean of 3 seladin-1-EGFP clones (A6, B1, B5)
B
caspase 3 activity (RFU)
1000
800
600
400
200
0
mean of 3 EGFP control clones (B5, B6, C1)
+Inhibitor
mean of 3 seladin-1-EGFP clones (A6, B1, B5)
+Inhibitor

Fig. 20

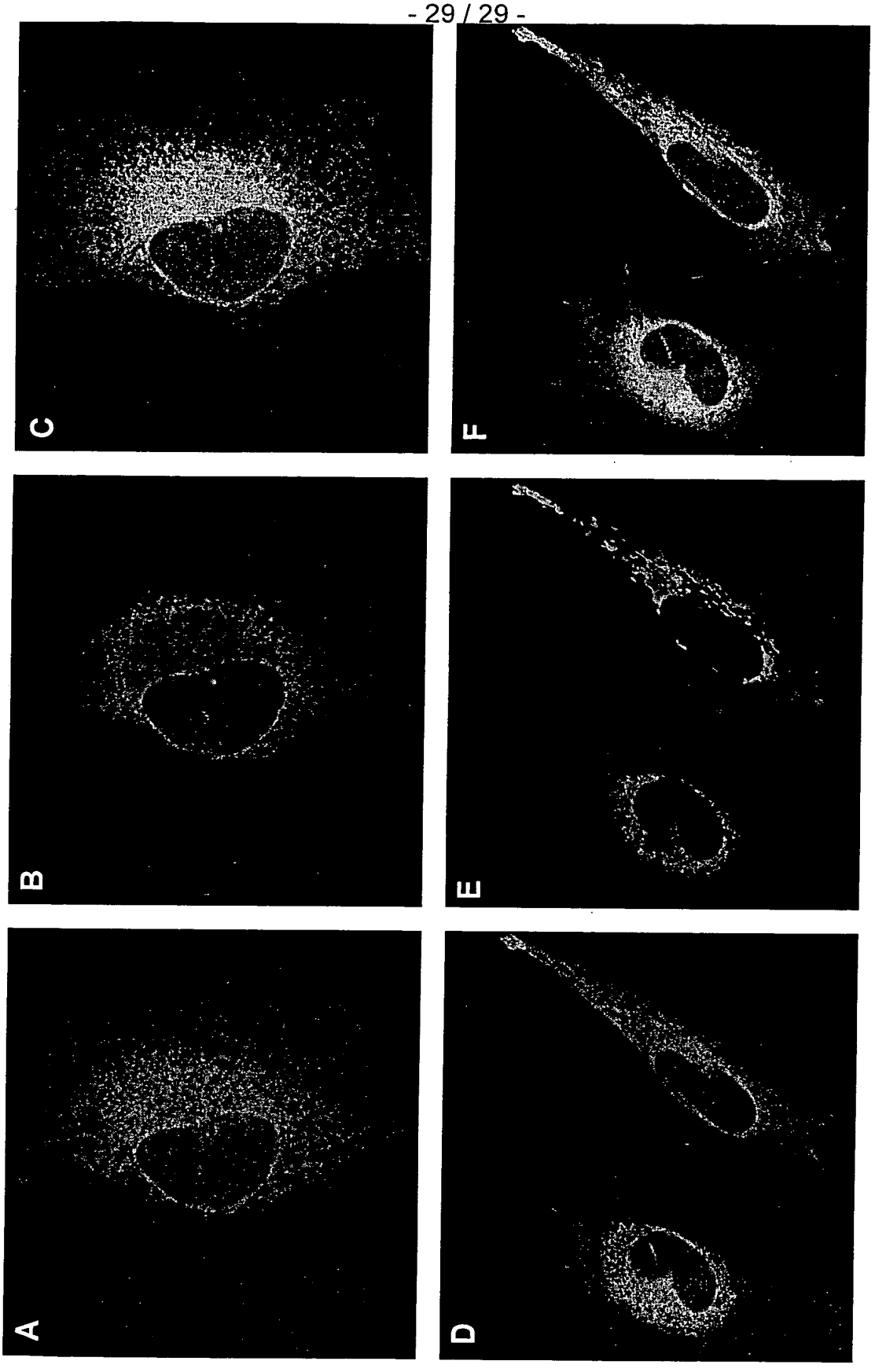

Fig. 21

PCT/EP00/05350